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Population and Community Dynamics of Four Species of *Pseudodactylogyrus* (Monogenea, Dactylogyridae) on Japanese Eel, *Anguilla japonica* (Temminck and Schlegel, 1846) Cultured in Two Chinese Fish Farms

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Abstract

The population and community dynamics of four monogeneans, *Pseudodactylogyrus anguillae*, *P. bini*, *P. microrchis*, and an unidentified *Pseudodactylogyrus* sp., on the gills of cultured Japanese eels, *Anguilla japonica* L., were investigated at two fish farms at Chaozhou, Guangdong Province, China. A total of 8,377 individual parasites were collected from sampling pond I (with more frequent chemical treatments for parasitic diseases: generally twice per month), including 338 *P. anguillae*, 7,388 *P. bini*, 491 *P. microrchis* and 160 *Pseudodactylogyrus* sp. At sampling pond II (with fewer chemical treatments for parasitic diseases: generally once during summer and winter months), 20,776 parasites were recovered, comprising 1,904 *P. anguillae*, 13,999 *P. bini*, 3,135 *P. microrchis* and 1,738 *Pseudodactylogyrus* sp. Monthly parasite community and population parameters, such as prevalence, mean abundance and species richness, varied irregularly and were not significantly correlated with water temperature at both fish farms. The infection parameters, however, exhibited a significant relationship with water temperature under indoor conditions. The diversity of monogenean communities in sampling pond II was higher than that in sampling pond I. These results may imply that the effects of water temperature exerted on the parasite population and communities were masked by human interference (drug implementation) in the fish ponds.

Keywords: *Pseudodactylogyrus* spp., dynamics, *Anguilla japonica*, famed ponds, China.

Introduction

Species of the monogenean genus *Pseudodactylogyrus* are cosmopolitan parasites of eels and often cause great economic losses of cultured eels in Asia (Ogawa and Egusa, 1976; Liu 2006), Europe (Buchmann, 1988*a*; Kennedy, 2007; Sasal *et al*., 2008), North America (Hayward *et al*., 2001) and Africa (Christison and Baker, 2007). To date, five species of *Pseudodactylogyrus* have been reported, i.e. *P. anguillae*, *P. bini P. microrchis*, *P. haze* and *P. kamegaii*. Numerous studies for *P. anguillae* and *P. bini* on their novel host, *Anguilla anguilla*, which is more susceptible than their original host, have been carried out (Buchmann, 1988a; 1988b; Matejusová *et al*., 2003; Arafa and Reda, 2012; Newbold *et al.*, 2015). However, their infection on the Japanese eel (*Anguilla japonica* L.), the original host, has received less attention (Fang *et al*., 2008).

Studies on the seasonal dynamics of populations and community structure of parasites have been conducted for several decades with many important findings (Køie, 1988; Nie and Kennedy, 1991; Boungou *et al*., 2008; Luo *et al*., 2010, Emre *et al*.,

2015). Many of them concerned the population dynamics of congeneric species of monogeneans on the same species of host (Simková *et al.*, 2000; Matejusová *et al.*, 2003; Boungou *et al.*, 2008), but only a few such studies have focused on the community dynamics of congeneric species (Knipes and Janovy, 2009; Muñoz and Randhawa, 2011) and none involved monogeneans on farmed Japanese eels. Previous studies on the parasite communities of eels mainly focused on endoparasitic helminths (Kennedy, 1993; 2001; Norton *et al.*, 2004), and most of these studies concentrated on community structure and composition (Gutiérrez, 2001). Only a few researches were conducted similarity analyses for communities in different sampling sites (Özer and Yılmaz, 2015).

It has been found that environmental factors, such as water temperature, play a significant role in the seasonal variation of parasite populations. Temperature is likely the most important abiotic factor influencing the population dynamics of monogeneans (Lambert, 1990). Many freshwater dactylogyrid monogeneans exhibit positive correlations with water temperature in either laboratory experiments (Fang *et al*., 2008) or field

investigations (Knipes and Janovy, 2009). In addition, chemical treatments of fish diseases, are common measures for preventing infectious diseases of cultured fishes, may result in environmental pollution, parasite resistance and host damage (Schmahl *et al.*, 1989; Iosifidou *et al.*, 1997) and even impact on the dynamics of parasite populations and communities.

The aims of the present study were: (1) to demonstrate the dynamic pattern of the monogenean parasites on cultured Japanese eels at both population and community levels; (2) to estimate the relationship between parasite infection levels and water temperature; and (3) to compare the dynamics of parasite populations and communities of cultured eels under different levels of human interference.

Materials and Methods

Field Collection

Field collections of monogeneans on Japanese eels were carried out monthly from May 2008 to May 2009 at two fish ponds belonging to two eel farms situated in different towns (Wenci, 23.769°N, 116.672°E; and Fengxi, 23.659°N, 116.602°E) in the Chaozhou Prefecture, Guangdong Province, China. Sampling pond I in the Wenci eel farm has an area of ~7,150 m^2 with an initial stocking number of 50,000 individual eels with a similar weight of ~0.033 kg; and sampling pond II in the Fengxi eel farm is ~3,000 m^2 and populated with 20,000 largish Japanese eels with a weight of between 0.156 and 0.567 kg.

A total of 260 Japanese eels from each sampling pond, 20 per month, were killed immediately after the determination of their length and weight. Gills of sampled fish were excised and examined under a stereo microscope for monogenean parasites. All parasites obtained were identified based on the shape and size of the haptoral sclerites and the size of the gonads under a light microscope (Olympus BX 41) equipped with phase contrast optics.

The main difference between the two sampling ponds is the frequency of chemical treatments for the parasitic diseases of the fish. Frequent and regular chemical treatments were implemented in sampling pond I (generally twice per month, mainly with mebendazole (2 mgL^{-1}) in the winter and spring months, and mebendazole (5 mgL^{-1}) and trichlorfon (1.5 mgL^{-1}) in the summer and autumn months), but there were fewer such treatments in sampling pond II (generally once a month only during summer and autumn months, with trichlorfon (1.5 mgL^{-1}).

Design of Indoor Experiments

Laboratory experiments were designed to test the effect of temperature on the infection level of *Pseudodactylogyrus* spp. on Japanese eels. During January 2011, a total of 170 adult Japanese eels (0.130–0.304 kg in weight, 44.7–60.6 cm in length) were purchased from an eel farm in Dongguan

prefecture, Guangdong Province, China. Except for 20 fish killed immediately to determine the background infection levels, the remaining 150 were divided randomly into 10 groups and cultured in 10 fish tanks each filled with 300 l of circulating water, i.e. there were 15 eels per tank. Based on the water temperature range in the sampling ponds, the temperatures in five pairs of tanks were set at 14°C, 18°C, 22°C, 26°C and 30°C, respectively, resulting in 30 eels at each water temperature. These fish were cultured in the laboratory for two months, during which time these parasites may have completed several generations according to the results of Fang *et al.* (2008), and were then killed to examine the monogeneans on the gills of each fish.

Data Analyses

Ecological terms, such as prevalence, mean abundance and mean intensity, were based on the definitions proposed by Bush *et al.* (1997). Prevalence, mean intensity and mean abundance were calculated monthly and the latter two parameters presented as the mean ± SD for each species. For parasite communities, the monogeneans on each individual host constitute an infracommunity, and all infracommunities during each sampling month comprise a component community (Bush and Holmes 1986). Species richness (average number of species per individual host) and diversity indices at both infracommunity and component community levels were calculated for each month. The Shannon-Wiener Index (H') was adopted as a measure of the diversity of the infracommunity and component community. Dominance was measured using the Simpson index (λ). Species richness (S) and Pielou's evenness index (J') were used to estimate the species composition and distribution at both infra- and component community levels.

One-way analysis of variance (ANOVA) was used to test the monthly variations of the difference in infracommunity parameters within and among the sampling months. The 2-sample Kolmogorov-Smirnov test was applied to evaluate differences in mean abundance between the two sampling ponds. Spearman's rho correlation analysis was employed to test the relationship between the mean abundances of *Pseudodactylogyrus* spp. and water temperature in the indoor culture experiment. Kruskal–Wallis tests were used to determine the significance of monthly variations in component community parameters. Except for the community parameters, which were calculated with Primer v6 (Clarke and Gorley, 2006), all these statistical analyses were performed with SPSS for Windows 13.0 at a significant level of 0.05.

RDA analysis was used to evaluate the significant differences between sampling sites based on species-temperature relationship using software CANOCO 5.0 (Ter Braak and Šmilauer, 2012). First, two data sets, species-sampling and temperature-sampling, were established which contained 26

samples, 4 species and temperature variable for outdoor investigations, 5 samples, 4 species and temperature variable for indoor experiments. Second, based on species-sampling data, the length of the species gradient were calculated using detrended correspondence analysis (DCA) with detrending by segments. Third, as the value of the length of the species gradient were 1.13 (< 2) for outdoor investigations and 1.23 (< 2) for indoor experiments, the RDA was performed to evaluate the strength of the relationship between the species abundances and temperature. Species abundances were square root transformed for all analyses. The significance of temperature and the first four axes were tested using 999 unrestricted permutations.

Based on the monthly mean abundances of each parasite species, MDS (non-metric multi-dimensional scaling) analysis was performed to determine the similarity between parasite communities in different months. The values for mean abundance were log (ln) transformed, followed by calculation of the Bray-Curtis similarity matrix which permits MDS analyses using the software Primer v6. The similarity level was set according to the similarity matrix.

Results

Seasonal Population Dynamics

A total of 8,377 individuals of *Pseudodactylogyrus* were collected from Japanese

eels in sampling pond I, including 338 *P. anguillae*, 7,388 *P. bini*, 491 *P. microrchis* and 160 *Pseudodactylogyrus* sp. In total, 20,776 specimens of *Pseudodactylogyrus* were obtained from sampling pond II, comprising 1,904 *P. anguillae*, 13,999 *P. bini*, 3,135 *P. microrchis* and 1,738 *Pseudodactylogyrus* sp. The number of parasites collected from sampling pond II was much greater than that collected from sampling pond I, and *P. bini* was the dominant species in both sampling ponds. The prevalence, mean intensity and mean abundance of the four species of parasite varied irregularly from month to month throughout the year in both sampling ponds. However, the mean abundances of the four parasite species had a common peak during October 2008 in sampling pond I, and during April 2009 in sampling pond II. (Tables 1 and 2).

In sampling pond I, the prevalence of *P. bini* exhibited the least fluctuation (SD=5.99) throughout the 13 months when compared with those of the other three species (*P. anguillae*: SD=16.15; *P. microrchis*: SD=21.68; *Pseudodactylogyrus* sp.:SD=9.67). Changes in prevalence were similar for *P. anguillae* and *P. microrchis* from May to November 2008, and for *P. microrchis* and *Pseudodactylogyrus* sp. from October 2008 to February 2009. In addition, the prevalences of these three species presented similar variations during the autumn months (Table 1). All four species of parasites reached their maximum mean abundance in October 2008. The mean abundance of *P. anguillae* decreased as the water temperature

Table 1. Infection indices of four species of *Pseudodactylogyrus* on the gills of *Anguilla japonica* in sampling pond I

Time	T (°C)	No. of fish	Host length (cm)	*P. anguillae*			*P. bini*		
				P(%)	MI± SD	MA± SD	P(%)	MI± SD	MA± SD
08.05	24.2	20	47.87±2.95	50	3.00±2.91	0.75±1.89	90	3.78±4.76	1.70±3.64
08.06	25.6	20	50.92±3.68	35	4.29±4.46	1.50±3.27	85	14.94±12.64	12.70±12.83
08.07	28.5	20	46.34±2.93	60	2.08±1.83	1.25±1.74	95	30.05±34.45	28.55±33.24
08.08	30.0	20	54.78±5.66	20	1.25±0.50	0.25±0.55	90	44.78±22.40	40.30±25.27
08.09	29.5	20	52.27±4.65	30	1.33±0.52	0.40±0.68	95	23.63±16.00	22.45±16.44
08.10	27.8	20	55.08±5.34	50	8.80±8.88	4.40±7.60	100	139.60±208.17	139.60±208.17
08.11	20.3	20	55.26±5.43	25	1.80±1.10	2.20±2.98	100	39.15±35.39	39.15±35.39
08.12	17.1	20	58.46±6.62	60	2.92±3.60	1.75±3.11	90	11.72±10.42	10.55±10.50
09.01	13.7	20	59.55±4.77	60	3.67±3.20	2.20±3.05	95	14.42±9.75	13.70±10.02
09.02	24.2	20	62.54±4.76	65	2.92±1.70	1.90±1.97	80	13.06±9.32	10.45±9.86
09.03	21.3	20	66.42±6.24	35	2.14±1.68	0.75±1.41	90	19.78±16.90	17.80±17.11
09.04	22.5	20	63.20±4.10	45	2.33±0.87	1.05±1.32	100	19.30±23.80	19.30±23.8
09.05	23.8	20	62.15±3.77	20	1.25±0.50	0.25±0.55	90	14.61±14.80	13.15±14.70

Time	T (°C)	No. of fish	Host length (cm)	*P. microrchis*			*Pseudodactylogyrus* sp.		
				P(%)	MI± SD	MA± SD	P(%)	MI±SD	MA±SD
08.05	24.2	20	47.87±2.95	70	1.00±0.00	0.35±0.49	30	1.00±0.00	0.10±0.31
08.06	25.6	20	50.92±3.68	45	3.11±2.76	1.50±2.37	35	2.14±1.21	0.63±1.16
08.07	28.5	20	46.34±2.93	80	3.75±2.46	3.00±2.68	35	2.00±1.15	0.63±1.16
08.08	30	20	54.78±5.66	60	3.50±2.75	2.10±2.73	30	1.33±0.52	0.32±0.58
08.09	29.5	20	52.27±4.65	30	1.33±0.52	0.35±0.67	20	1.00±0.00	0.16±0.37
08.10	27.8	20	55.08±5.34	70	18.14±21.31	12.70±19.59	45	5.89±5.78	2.68±4.93
08.11	20.3	20	55.26±5.43	35	3.14±2.54	1.10±2.10	20	1.50±0.58	0.26±0.65
08.12	17.1	20	58.46±6.62	40	1.50±0.76	0.60±0.88	30	2.00±1.26	0.47±1.02
09.01	13.7	20	59.55±4.77	10	1.00±0.00	0.11±0.32	10	1.50±0.71	0.11±0.46
09.02	24.2	20	62.54±4.76	50	1.00±0.67	0.85±0.99	35	1.29±0.76	0.42±0.77
09.03	21.3	20	66.42±6.24	15	1.33±0.58	0.20±0.52	40	1.38±0.52	0.53±0.77
09.04	22.5	20	63.20±4.10	30	1.83±0.75	0.55±0.94	40	1.63±0.74	0.58±0.90
09.05	23.8	20	62.15±3.77	60	2.00±1.76	1.20±1.67	25	1.80±0.84	0.41±0.87

Note:Abbreviations: temperature (T), number (No.), prevalence (P), mean intensity (MI), mean abundance (MA), standard deviation (SD)

increased from June to September 2008 and increased when the temperature decreased from December 2008 to February 2009 (winter months). *P. bini* and *P. microrchis* had higher mean abundances during the summer months than during the remaining months, except for October 2008. The mean abundance of *Pseudodactylogyrus* sp. decreased during the summer and winter months, but increased from December 2008 to January 2009 (spring months) (Table 1).

In sampling pond II, the prevalence of all four species of parasites followed similar, irregular patterns, except for *P. microrchis,* which exhibited different dynamics from October to December 2008. During the winter months, the prevalence of *P. bini* and *P. anguillae* tended to increase, whereas those of *P. microrchi*s and *Pseudodactylogyrus* sp. decreased from December 2008 to January 2009 and then increased sharply in February 2009. The mean abundance of *P. anguillae* was relatively higher during the winter months (December 2008, January and February 2009), with the highest level in April 2009. *P. microrchis* and *Pseudodactylogyrus* sp. reached their greatest mean abundance in April 2009, with distinctly lower and less variable values during other months (Table 2). As for *P. bini*, its highest mean abundance values were observed in May,

August and December 2008. (Table 2).

Relationship Between Species Abundances and Water Temperature

In sampling pond I, the water temperature had a significant positive relationship with the mean abundance of *P. bini* (r=0.767, P=0.010) and *P. microrchis* (r=0.730, P=0.017), but was not correlated significantly with the mean abundances of *P. anguillae* (r=0.339, P=0.338) and *Pseudodactylogyrus* sp. (r=0.543, P=0.105). In sampling pond II, no significant relationship was detected between water temperature and the mean abundance of any species, except for *P. anguillae* (r=-0.637, P=0.048) which was negative.

On indoor conditions, comparing with the control group, the infection level of parasites increased at 14℃ and 18℃ for *P. anguillae*, *P. microrchis* and *Pseudodactylogyrus* sp., which decreased at all temperature groups for *P. bini* (Table 3). *P. anguillae*, *P. microrchis* and *Pseudodactylogyrus* sp. achieved their highest infection at 14℃ and decreased as the temperature increased, which were rare when the temperature arrived at 30℃. Whereas, *P. bini* arrived its peak at

Table 2. Infection indices of four species of *Pseudodactylogyrus* on the gills of *Anguilla japonica* in sampling pond II

Time	T (°C)	No. of fish	Host length (cm)	*P. anguillae*			*P. bini*		
				P(%)	MI± SD	MA± SD	P(%)	MI± SD	MA± SD
08.05	24.0	20	58.72±3.36	65	7.08±7.91	4.60±7.18	85	111.41±85.63	94.70±88.55
08.06	25.0	20	59.29±2.96	75	5.07±5.96	3.80±5.59	90	30.06±25.23	29.20±25.87
08.07	28.2	20	60.87±3.05	60	4.92±3.45	2.95±3.61	65	86.31±46.73	56.10±57.84
08.08	29.7	20	58.45±2.30	80	5.75±3.71	4.89±3.96	85	159.41±122.95	135.50±127.04
08.09	29.1	20	59.88±3.02	0	0.00±0.00	0.00±0.00	75	10.07±10.82	7.55±10.31
08.10	26.3	20	66.82±3.93	55	5.00±6.22	2.25±4.78	70	106.71±102.47	74.70±98.5
08.11	21	20	58.53±3.26	45	5.30±12.26	2.40±8.41	50	31.10±50.81	15.55±38.44
08.12	16.9	20	62.90±3.52	65	30.15±66.26	19.6±54.69	85	153.82±135.05	130.75±136.14
09.01	13.9	20	61.81±3.68	80	14.06±14.38	14.75±20.25	95	16.68±14.63	15.00±15.42
09.02	23.8	20	63.36±3.81	90	14.06±13.61	12.65±13.59	95	27.63±30.88	26.25±30.68
09.03	21.7	20	59.59±4.96	55	5.18±6.32	2.85±5.29	80	42.06±50.34	33.65±47.94
09.04	22.4	20	54.74±5.54	75	32.47±37.51	24.35±35.28	80	85.25±133.76	68.2±123.89
09.05	23.5	20	59.77±3.74	55	7.09±6.76	4.65±5.88	65	18.38±23.80	11.95±20.94

Time	T (°C)	No. of fish	Host length (cm)	*P. microrchis*			*Pseudodactylogyrus* sp.		
				P(%)	MI± SD	MA± SD	P(%)	MI± SD	MA± SD
08.05	24.0	20	58.72±3.36	50	9.18±8.40	5.05±7.69	30	3.00±1.67	0.90±1.65
08.06	25.0	20	59.29±2.96	90	12.41±10.46	10.65±10.54	65	3.00±2.61	1.95±2.54
08.07	28.2	20	60.87±3.05	75	19.20±9.78	14.40±11.97	65	7.92±5.04	5.15±5.58
08.08	29.7	20	58.45±2.30	80	7.75±7.33	6.20±7.24	60	2.67±0.89	1.40±1.43
08.09	29.1	20	59.88±3.02	30	2.17±2.86	0.65±1.79	0	0.00±0.00	0.00±0.00
08.10	26.3	20	66.82±3.93	60	7.75±98.39	4.65±8.14	40	5.63±4.00	2.25±3.73
08.11	21	20	58.53±3.26	70	3.50±3.50	2.45±3.33	30	6.33±9.54	1.90±5.73
08.12	16.9	20	62.90±3.52	55	7.55±6.47	4.15±6.07	40	10.38±16.06	3.65±11.00
09.01	13.9	20	61.81±3.68	20	2.50±1.30	0.50±1.15	20	1.25±0.50	0.25±0.55
09.02	23.8	20	63.36±3.81	40	1.38±0.52	0.55±0.76	70	3.36±2.44	2.35±2.56
09.03	21.7	20	59.59±4.96	50	8.20±11.40	4.10±8.90	45	7.33±11.53	3.30±8.37
09.04	22.4	20	54.74±5.54	75	134.00±209.03	100.5±189.05	80	75.00±71.03	60.60±101.26
09.05	23.5	20	59.77±3.74	60	4.83±8.71	2.90±3.73	75	4.55±3.86	2.50±3.63

Note:Abbreviations: temperature (T), number (No.), prevalence (P), mean intensity (MI), mean abundance (MA), standard deviation (SD)

Table 3. Infection indices of four species of *Pseudodactylogyrus* on the gills of *Anguilla japonica* in indoor conditions

T (°C)	NO. of fish	Host length (Mean± SD)	*P. anguillae*						*P. bini*			*P. microrchis*			*Pseudodactylogyrus* sp.		
			P (%)	MI± SD	MA± SD	P (%)	MI± SD	MA± SD	P (%)	MI± SD	MA± SD	P (%)	MI± SD	MA± SD	P (%)	MI± SD	MA ± SD
14	29	51.84±2.87	93.1	4.42±3.73	3.97±3.77	34	1.10±0.32	0.38±0.56	83	3.46±2.55	2.86±2.67	93	6.56±4.42	6.10±4.42			
18	30	50.33±2.00	13	8.75±7.80	1.03±3.67	47	4.08±3.88	3.27±3.83	27	5.13±5.27	1.37±3.47	30	4.56±4.39	1.37±3.13			
22	30	51.81±2.78	10	1.67±0.58	0.17±0.53	60	7.11±10.69	4.27±8.92	27	3.63±4.31	0.97±2.67	10	7.33±5.86	0.73±2.72			
26	30	52.33±2.70	10	2.00±0.00	0.10±0.31	73	6.36±4.60	3.73±4.57	20	2.80±1.30	0.37±0.94	17	1.60±0.90	0.27±0.69			
30	29	52.94±3.70	3	1.00±0.00	0.30±0.00	48	6.00±2.40	2.90±0.48	10	1.00±0.00	0.10±0.31	0	0.00±0.00	0.00±0.00			
Control	20	53.26±3.68	65	1.40±0.55	0.88±0.83	70	9.67±5.89	7.25±6.69	75	1.43±0.79	1.25±0.89	75	1.43±0.53	1.25±0.71			

Note:Abbreviations: temperature (T), number (No.), prevalence (P), mean intensity (MI), mean abundance (MA), standard deviation (SD)

26℃, of which infection were higher in relatively high temperature groups (26℃ and 30℃) than those in low temperature groups (14℃ and 18℃). Accordingly, mean abundance was positively correlated with water temperature for *P. bini* (r=0.262, P=0.002). For the other three species, in contrast, their mean abundances were significantly negatively correlated with water temperature (*P. anguillae*: r=−0.594, P=0.000; *P. microrchis*: r=−0.478, P=0.000; *Pseudodactylogyrus* sp.: r=−0.648, P=0.000).

With respect to RDA, no outerliers were revealed. The relationship between temperature and species abundances was not significant based on data collected in outdoor investigation (p-value =0.327) and significant (p-value =0.035) based on indoor data. The cumulative explained fraction of the variance in the first two axes and the explanatory variable (temperature) accounted for the total variation were 76.12% and 4.21%, 83.47% and 56.07% for RDA of outdoor and indoor data respectively (Figure 1). As for the RDA based on indoor data, the first RDA axis is most strongly influenced by temperature (correlation = −0.97). *P. bini* owned its highest score on the negative side of the first axis, showing its tendency towards higher temperature groups. In contrast, *P. anguillae*, *P. microrchis* and *Pseudodactylogyrus* sp. showed higher abundances when temperature is lower (Figure 1b).

Seasonal Dynamics of the Monogenean Communities

At the infracommunity level, the species richness ranged from 1-4, with the maximum mean values in February and April 2009, respectively, for sampling ponds I and II, and their lowest mean values in November and September 2008 (Figure 2a). The highest mean values of the Shannon-winner Index were during the spring months (May 2008 for sampling pond I, April 2009 for sampling pond II), and the lowest values occurred in the autumn months (November 2008 for sampling pond I, September 2008 for sampling pond II) (Figure 2d). The monthly variations of the Simpson index revealed that infracommunities exhibited their highest levels of dominance during November 2008 and September 2008 in sampling ponds I and II, respectively, whereas their lowest levels were in May 2008 and April 2009 (Figure 2b). A significant difference for

Pielou's evenness index was observed between the two sampling ponds (Z=-3.468, P=0.001). Parasites on the eels collected from sampling pond I were more evenly distributed than those from sampling pond II. All community parameters were significantly different during the sampling months in both sampling ponds (P<0.05).

At the component community level, *P. bini* was the dominant species, accounting for >50% of the entire community throughout the year, except for April 2009 in sampling pond II (Figure 3). Species richness was stable at 4 throughout the sampling period, except for one low value of 2 in sampling pond II during August 2008 (Figure 4a). The Shannon-Wiener's Index of diversity *(H')* varied irregularly for both sampling ponds. In sampling pond I, it fell to its lowest levels during September (0.22) and November (0.23) 2008 and ascended to its peak in May 2008 (1.07). In the case of sampling pond II, the lowest value of *H'* (0.28) appeared in September 2008 and the highest (1.29) in April 2009 (Figure 4d). Based on the Kruskal–Wallis test (P>0.05), none of these community parameters exhibited significant monthly differences. For the monogenean component communities on the cultured eels from different sampling ponds, Pielou's evenness index showed them to be significantly different (Z=-2.231, P=0.026).

MDS analysis revealed that the monogenean component communities on the gills of cultured Japanese eels during different months shared over 70% and 50% similarities in sampling ponds I and II, respectively. At the 90% similarity level, component communities in the same season tended to group together, as in the cases of March, April and May 2009 in sampling pond I and January and February 2009 in sampling pond II. The component community during May 2008 in sampling pond I (Figure 5a) and that during April 2009 in sampling pond II (Figure 5b) deviated from months on either side, which were generally grouped together. Considering all 26 component communities together, all had a >50% similarity, but those from the different sampling ponds grouped separately at the 80% similarity level (Figure 5c).

Discussion

In the present study, the mean abundances of four species of monogeneans on cultured Japanese eels

eels, *Anguilla japonica,* did not exhibit regular seasonal variation in two sampling ponds subjected to a different frequency of drug implementation. Similar results were also reported for *Cichlidogyrus* spp. parasitizing *Oreochromis niloticus* in Burkina Faso (Boungou *et al*., 2008). Regular or predictable

seasonal parasite population dynamics have previously been demonstrated in relatively open waters, such as reservoirs (Nie, 1996), lotic waters (Appleby and Mo, 1997) and an estuary (Winger *et al*., 2008), and also under experimental conditions (Scott and Anderson, 1984).

a. RDA with outdoor data   b. RDA with indoor data
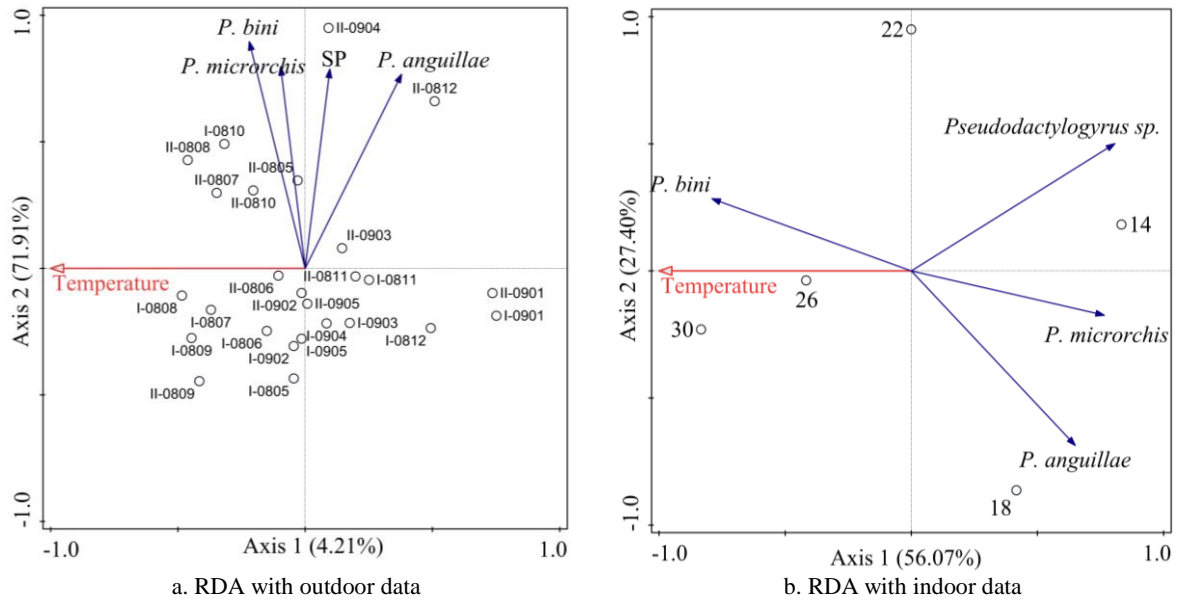
Figure 1. Triplot diagrams of redundancy analysis (RDA) presenting the relationship between temperature and species abundances based on data collected from outdoor investigation (a) and indoor experiments (b).

a. Species richness (*S*)

b. Simpson index (*λ*)

c. Pielou's evenness index (*J'*)

d. Shannon-Wiener Index (*H'*)
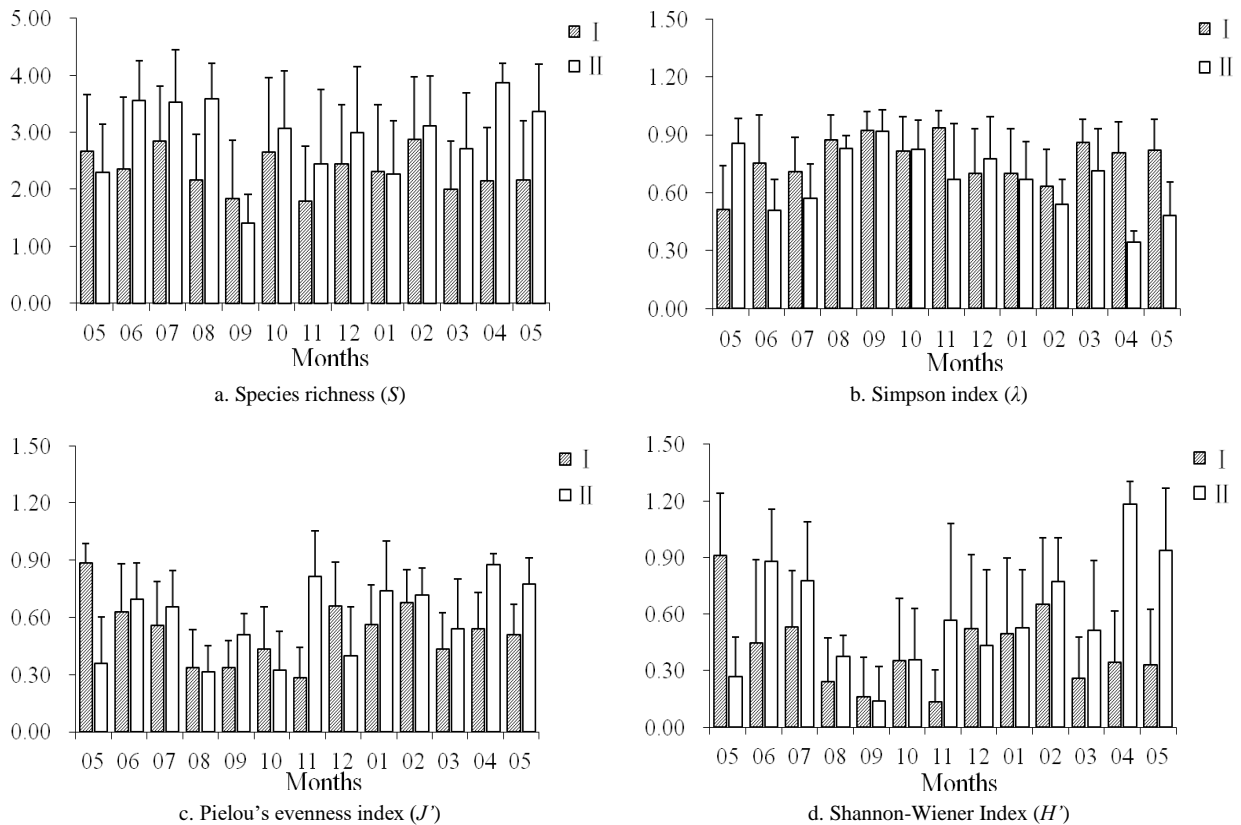
Figure 2. Monthly parameters of monogenean communities at the infracommunity level on *Anguilla japonica* in sampling ponds I and II located in Chao Zhou, Guangdong Province, China.
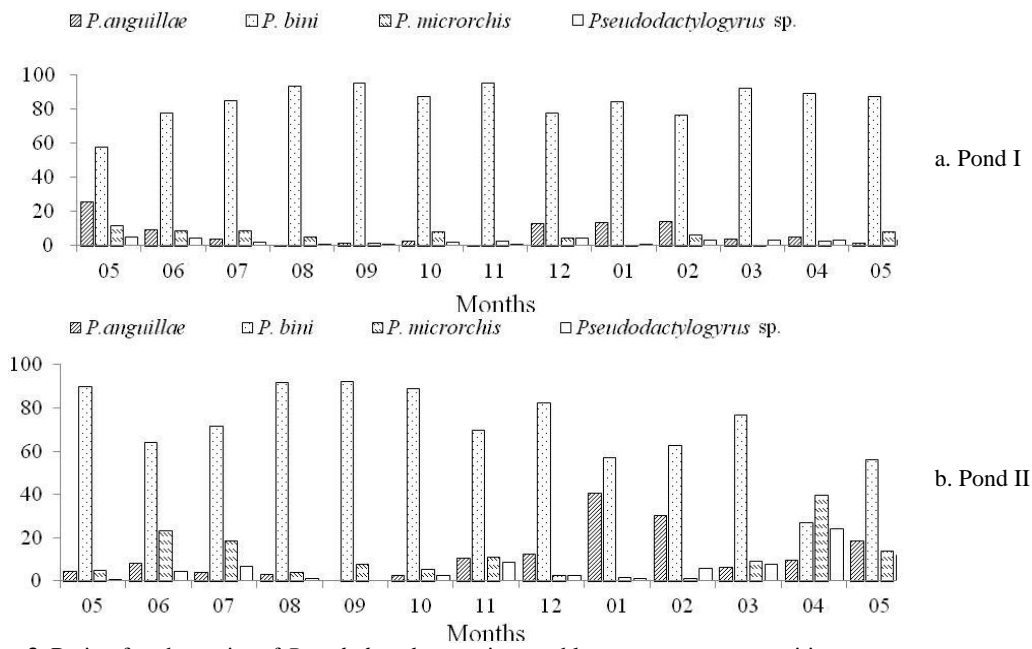
Figure 3. Ratio of each species of *Pseudodactylogyrus* in monthly component communities.
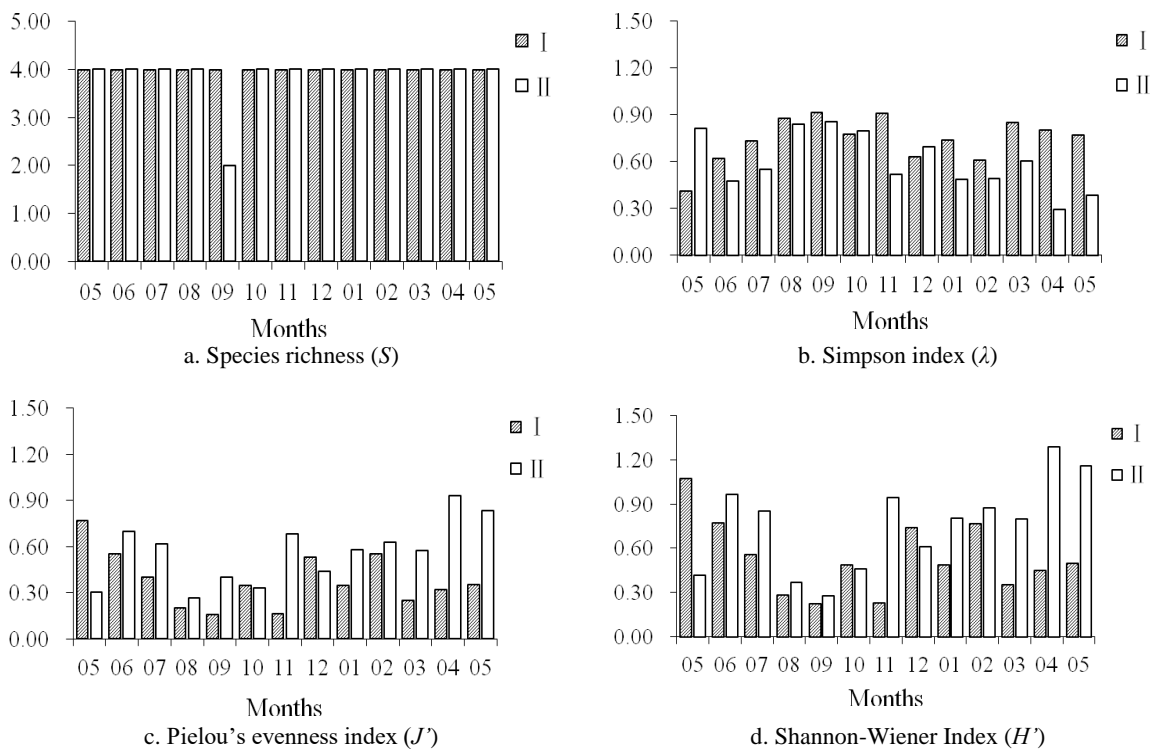

Figure 4. Monthly parameters of monogenean communities at the component community level on *Anguilla japonica* in sampling ponds I and II located in Chao Zhou, Guangdong Province, China.

Temperature is generally considered to be the most important factor associated with seasonal variation in monogenean infections, because all stages of the monogenean life cycle (oviposition, embryogenesis, oncomiracidium, postlarval development and adult life span) are temperature-dependent (Buchmann, 1988*b*, 1990*a*; Tubbs *et al.*,

2005; Hirazawa *et al.*, 2010). Indoor experiments in the present study have demonstrated that infection levels of *Pseudodactylogyrus* spp. are temperature-dependent. Specifically, *P. bini* is a eurythermal but thermophilic species with an optimal water temperature of (26~30°C), whereas the mean abundance of the other three species of
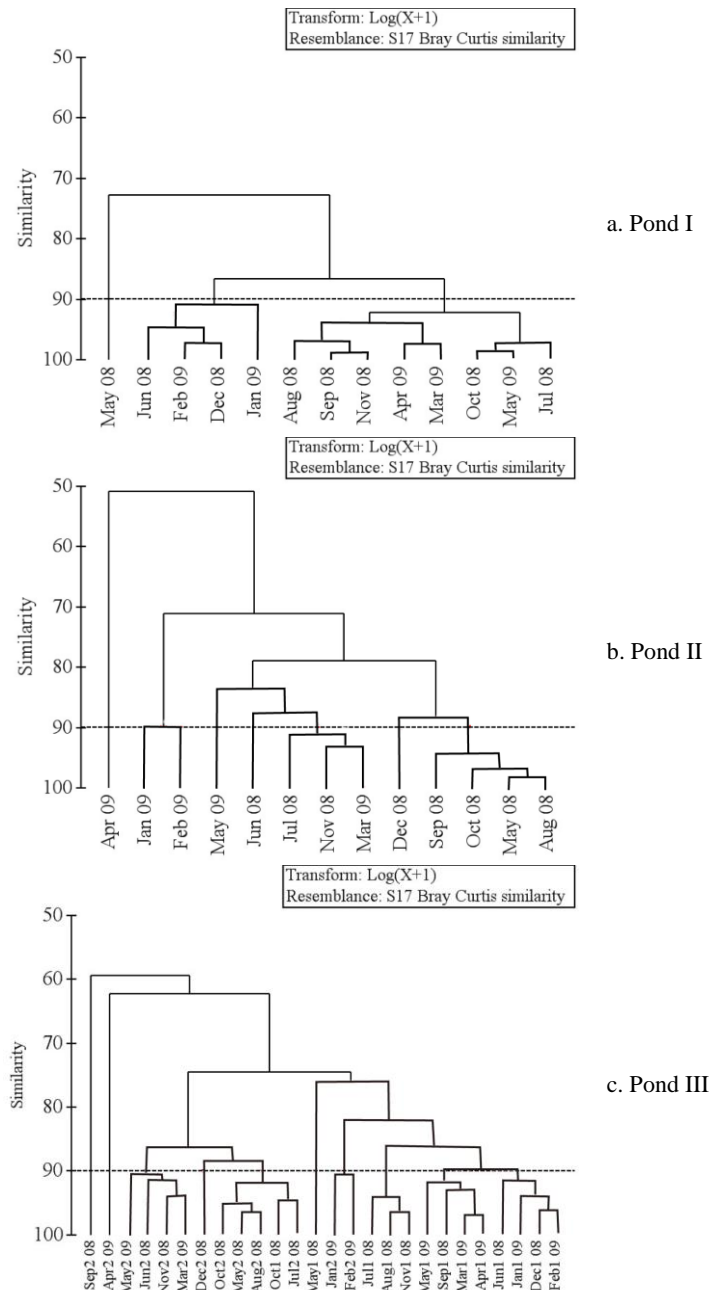
Figure 5. Cluster dendrogram of component communities on the host *Anguilla japonica.*

Pseudodactylogyrus decreased with increasing water temperature in the range of (14~30°C). The thermophily of *P. bini* was also reflected in the significant positive correlation between its mean abundance and water temperature in the sampling ponds. Similar results on the relationship between *Pseudodactylogyrus* infection levels and water temperature have also been reported by Køie (1988), Nie and Kennedy (1991) and Buchmann (1997). However, with the exception of *P. bini,* such a significant relationship between water temperature and either mean abundance or prevalence was not apparent for the other 3 species of *Pseudodactylogyrus* in the present study over a one-year survey. RDA revealed that the infection of

Pseudodactylogyrus were significantly temperature-dependent on experimental conditions. Specifically, *P. bini* was positively related to temperature while the other three species were negatively at the range of (14~30°C). However, the same results calculated by RDA were not represented on conditions of sampling ponds. Similarly, *Dactylogyrus extensus* in Özer's (1999) study showed highest intensity at 16°C in the farm but 12°C in the lake. Such differences can be ascribed to the complicated aquatic environments in the fish ponds and the chemical treatment of the ponds for disease control. On the one hand, the complicated environment of these ponds which was considered as totally confined system may weaken the impact exerted from temperature. Water chemical

variables such as pH, stream flow affected the living and transmission of oncomiracidia (Baker, 2000) and low oxygen saturation was responsible to inhibit the embryonation of eggs (Buchmann, 1988b). McCcammon (2010) observed that *Periclimenes pedersoni,* a cleaner shrimp, had a strong effect on the abundance and size of parasitic monogenean *Neobenedenia melleni* on a host reef fish. Buchmann's (1994) experiments suggested that the free living fauna serving as egg predators were liable to depress the infection level and numerous bacteria might damage the parasite tegument as well. On the other hand, a large number of chemicals including medium-chain fatty acids (Wang *et al.*, 2009), praziquantel, levamizole, bunomidine (Buchmann *et al.*, 1990b), mebendazole (Li *et al.*, 1997; Buchmann and Bjerregaard., 1990c), potassium permanganate, trichlorfon (Umeda *et al.*, 2006), have been reported to have anthelmintic efficacy on removal of *Pseudodactylogyrus* spp. from European eels, some of which have become regular pesticides in fishery for years. In our investigations, anthelmintic drugs are indispensable for farm management. In sampling pond I, anthelmintic drugs were applied every month, whereas, in sampling pond II, drugs were applied only when the water temperature was high. Thus, drug disturbance may account for the different trends of monogenean infections between the two sampling sites. It is a regret that we haven't got more environment variables in the present study. In addition, our limited samples of fish (20 per month) may also be a reason for the irregular seasonal pattern in parasite population on the gills of cultured Japanese eels and their negligible relationship with temperature.

At the infracommunity level, the species richness of the monogeneans on the cultured eels varied from zero to four in different months, but it remained as four at the component community level in both sampling ponds, which (with a low species diversity and dominated by a single species) is similar to the parasite community of European eels studied by Kennedy *et al.* (1992). The other three parameters of parasite infracommunities and component communities presented similar seasonal dynamics, i.e. their peaks and troughs appeared during similar months in the two sampling ponds. This seasonality was also reflected in the result of the MDS analyses, which showed that the component communities in months of the same season were generally clustered together. Despite this seasonality, all component communities in different months had >50% similarity for each sampling pond, indicating a comparatively stable parasite community on cultured eels, which is different from that found by Muñoz and Randhawa (2011) for parasite communities of the intertidal fish *Scartichthys viridis*.

As for the separate grouping of parasite component communities from the different sampling ponds by MDS analyses, this can be attributed to a significant difference in species evenness. *Pseudodactylogyrus bini* developed greater number in relatively high (22°C~30°C) temperature conditions, while the rest with large hamuli own their optimal temperature for reproductivity in lower water temperature (14°C~22°C). Despite temperature predilection, *P. bini* kept being dominant species through all seasonal communites in both sampling sites. This may due to its strong tolerance to host immune reaction (Buchmann and Lindenstrøm, 2002) and stronger resistance to drugs than the other three sepecies studied here. Therefore, species evenness will be high in farm with less drug implementation, such as sampling pond II in our research. However, this is not consistent with Ibrahim's (2012) results in terms of the parasite community on cultured *Tilapia zillii,* which exhibited a higher species richness and diversity than that on wild hosts. This inconformity may be ascribed to a smaller difference between the two sampling ponds in the present study than that between the cultured pond and the natural environment in Ibrahim's (2012) study.

Acknowledgements

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